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Thrombolysis With Recombinant Unglycosylated Single-Chain Urokinase-Type Plasminogen Activator (Saruplase) in Acute Myocardial Infarction: Influence of Heparin on Early Patency Rate (LIMITS Study)

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Objectives. **The Liquemin in Myocardial Infarction During Thrombolysis With Saruplase (LIMITS) study was instituted to evaluate and characterize the effect of a prethrombolytic heparin bolus (5,000 IU) on the efficacy and safety of saruplase in patients with acute myocardial infarction.**

Background. **Heparin has been used after thrombolytic therapy for acute myocardial infarction to prevent reocclusion of the infarct-related artery.**

Methods. **The study was designed as a randomized, parallel-group, double-blind, multicenter trial. Patients were treated within 6 h of onset of symptoms with either a bolus of 5,000 IU of heparin (Liquemin) (n = 56, HSH group) or placebo (n = 62, PSH group) before thrombolytic treatment with saruplase given as a 20-mg bolus followed by an infusion of 60 mg over 60 min. Thirty minutes after completion of thrombolysis, an intravenous heparin infusion was administered for 5 days. Before coronary angiography was performed at 6 to 12 h after start of lysis, an additional bolus of 5,000 IU heparin was given to all patients. End points studied were patency of the infarct-related artery, changes in the hemostatic system and bleeding complications.**

Results. **In the HSH group (heparin–saruplase–heparin), 78.6% of patients had an open infarct-related vessel (Thrombolysis in Myocardial Infarction [TIMI] flow grade 2 or 3) compared with 56.5% in the PSH group (placebo–saruplase–heparin) (intention-to-treat analysis, p = 0.01). No significant difference was observed between the two groups with regard to changes in fibrinogen and fibrin/fibrinogen degradation products. A total of eight bleeding complications (14.3%) were observed in the HSH group and five (8.1%) in the PSH group; no cerebrovascular event occurred, and no allergic reaction was reported. A total of 12 patients died during the hospital stay, 3 in the HSH group (5.4%) and 9 in the PSH group (14.5%).**

Conclusions. **In acute myocardial infarction, the administration of a heparin bolus before thrombolytic therapy with saruplase is associated with a significantly higher patency at angiography 6 to 12 h after the start of thrombolysis without any appreciable increase in risk of bleeding.**

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Heparin has been used empirically after thrombolytic therapy for acute myocardial infarction to prevent thrombotic reocclusion of the infarct-related coronary artery. The optimal strategy of heparin administration has not yet been determined. The timing and dose of heparin has varied widely in clinical trials but has been used as adjunctive therapy with all thrombolytic agents (i.e., recombinant tissue-type plasminogen activator [alteplase], streptokinase, anistreplase and urokinase) in the setting of acute myocardial infarction (1–7). Saruplase, a recombinant single-chain urokinase-type plasminogen activator, has been tested in clinical trials (8–15) (INN [a full-length unglycosylated single-chain urokinase-type plasminogen activator prepared from recombinant *Escherichia coli*]) (Fig. 1). In the current randomized, double-blind trial, an intravenous bolus of heparin or placebo was given immediately before thrombolysis with saruplase. Thirty minutes after the saruplase infusion was completed a continuous intravenous infusion of heparin was administered to all patients for 5 days and was titrated to maintain thrombin time at two to three times the control value. The trial was designed to evaluate the effect of adjunctive heparin on coronary patency, incidence of bleeding complications and hemostasis after saruplase therapy in patients with acute myocardial infarction.

*Gruenenthal GmbH, Aachen, Germany. A complete list of participating centers and investigators for the LIMITS Study appears in the Appendix. Saruplase was provided by Gruenenthal GmbH, Aachen, Germany.

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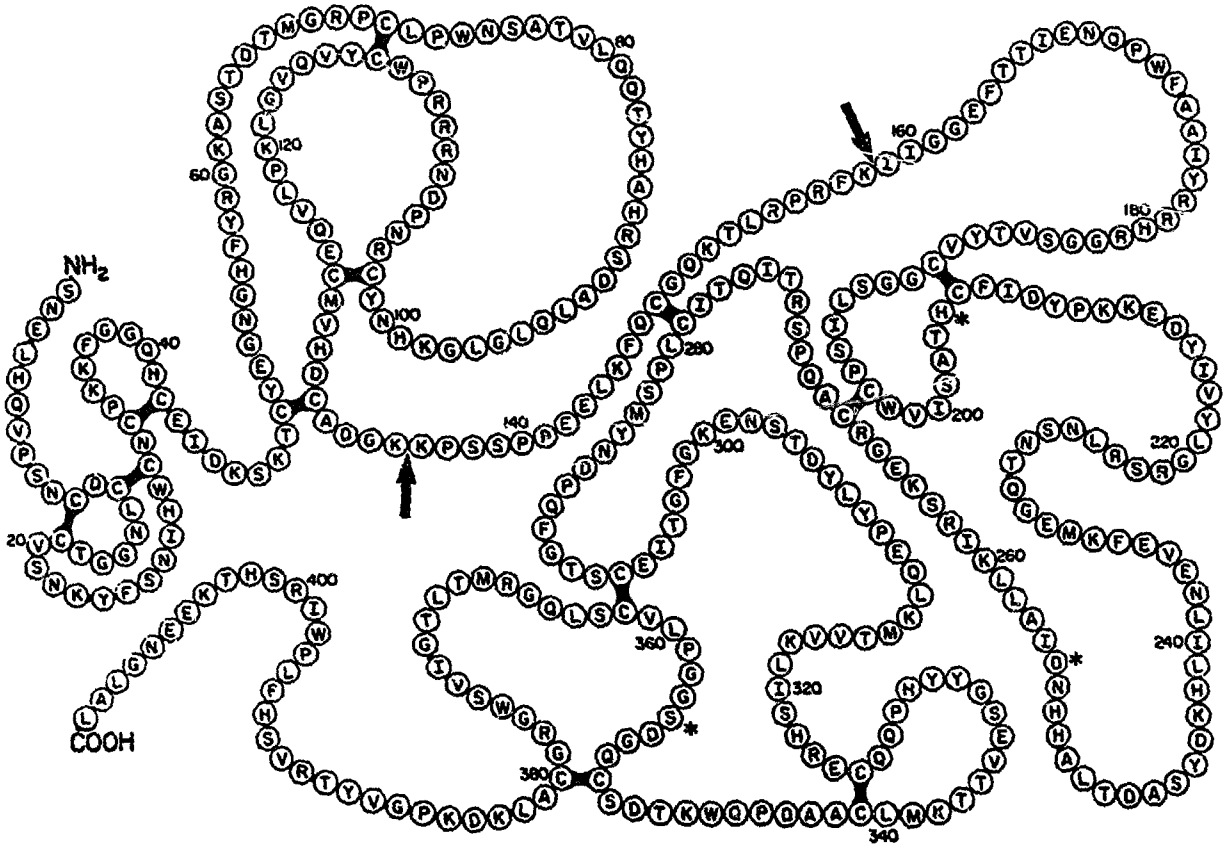

Figure 1. Schematic presentation of amino acid sequence of saruplase, single-letter code. **Asterisks** = active-site amino acids; **arrows** = cleavage sites into active high molecular weight two-chain urokinase-type plasminogen activator derivative (Lys 158) and low molecular weight derivative (Lys 135).

Methods

The study was a randomized placebo-controlled, parallel-group, double-blind trial. Fifteen local hospitals in proximity to the University Clinic of Göttingen, Germany, participated in the trial. The study protocol was approved by the ethics committee of the University of Göttingen as well as a central ethics and advisory board, which was specially convened for the study. All patients gave informed consent before entry into the study. The administration of trial medication was taken as the irrevocable entry of a patient into the study.

Inclusion criteria. Patients 20 to 75 years old with suspected acute myocardial infarction characterized by chest pain lasting for at least 30 min despite oral treatment with nitrates or calcium antagonists, or both, were screened for entry. ST segment elevation of 0.3 mV in at least two precordial leads or ST segment elevation of 0.2 mV in at least two limb leads, or both, had to be present. Patients with a first or a previous infarction in a remote area were included. The interval between onset of symptoms and start of infusion was not to exceed 6 h.

Exclusion criteria. The currently accepted contraindications for thrombolytic therapy were used as exclusion criteria. In addition, patients who refused consent for coronary angiography, were obese or had cachexia (Broca index <70 or >130), administration of heparin in the previous 24 h, anticoagulation, cardiogenic shock, traumatic resuscitation, complete left bundle branch block, a cardiac pacemaker, previous coronary artery bypass surgery, endocarditis, acute pericarditis or atrial fibrillation of any origin were excluded.

Study treatment. Unless contraindicated all patients were started on a nitroglycerin (3 mg/h) infusion, which was titrated according to blood pressure and continued until oral nitrates could be given. One group of patients received a single intravenous bolus of 5,000 IU of heparin as premedication in blinded manner (heparin–saruplase–heparin [HSH] group). The second group of patients was given a corresponding placebo bolus (placebo–saruplase–heparin [PSH] group) (Fig. 2). Thereafter, all patients immediately received a bolus of 20 mg of saruplase, followed by an intravenous infusion of 60 mg of saruplase over 60 min. An intravenous infusion of heparin (15 IU/kg per h) was started in all patients 30 min after completing the saruplase infusion and continued until the end of the fifth day after thrombolytic treatment. An additional

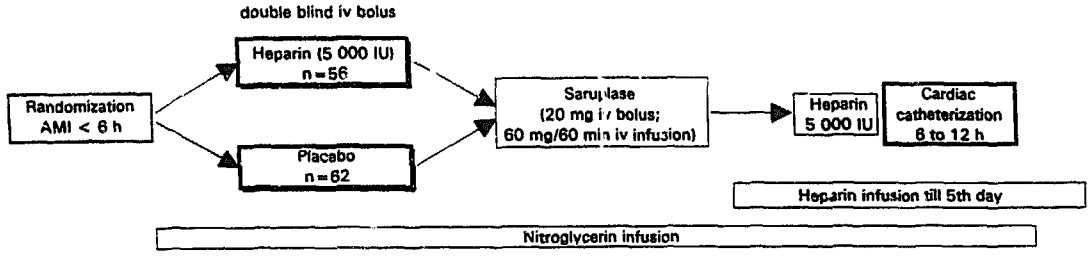

Figure 2. Action and therapy scheme. AMI = acute myocardial infarction; iv = intravenous.

intravenous bolus of 5,000 IU of heparin was given before catheterization. Antiplatelet drugs were not allowed between start of thrombolysis and the end of the fifth day. Other medication could be given if necessary. Saruplase was prepared by Gruenenthal GmbH, Aachen, Germany and provided in vials containing 10 mg of lyophilized active compound plus stabilizing ingredients. Heparin (Liquemin 5000) was purchased from Hoffmann-La Roche AG, Grenzach-Wyhlen, Germany. A corresponding matching placebo was manufactured by Gruenenthal ensuring that the study could be performed in blinded manner.

Coronary angiography. After completion of the thrombolytic infusion patients were transferred from local hospitals to the catheterization laboratory in Göttingen so that coronary angiography could be performed between 6 and 12 h after the start of thrombolytic therapy. Standard projections were used, and each main coronary artery was viewed three times. Biplane ventriculography was also performed. Additional procedures, such as percutaneous transluminal coronary angioplasty or intracoronary lysis, were not allowed before visualization of the infarct-related artery. Qualitative assessment of the flow in the infarct-related artery was performed according to the TIMI grading system (16). The films were read by investigators who had no knowledge of the treatment: TIMI flow grades 2 and 3 were considered patent and 0 and 1 occluded. An estimation of the degree of stenosis of the infarct-related artery was recorded.

Hematologic studies. Blood was sampled through an indwelling needle used exclusively for this purpose. Blood samples were taken on admission and 60 min after starting the study medication. A special kit was provided to ensure uniform handling of samples. The first 2 ml of blood was discarded. After collection of the second sample, both samples were centrifuged, and the plasma was frozen. The concentrations of fibrinogen, fibrinogen degradation products, fibrin degradation products and thrombin–antithrombin III complex were assayed centrally. Routine laboratory variables were documented at admission and on the following day.

Determination of infarct-specific enzyme activity. Venous blood samples were taken before commencing thrombolytic therapy. Starting 60 min after the end of the saruplase infusion, eight samples were to be taken at 2-h intervals up to 16 h, and a further eight samples were to be taken at 4-h intervals up to 48 h. The samples were sent to the University Hospital of Göttingen for central assay of the activity of creatine kinase-MB isoenzyme (CK-MB).

Adverse events, electrocardiography and follow-up. Adverse events were reported as "bleeding complications" or "cardiovascular events" or "other adverse events." The severity of the event was evaluated using predefined criteria. The time and date of onset and duration, as well as the treatment required, were documented. Where possible, computer-assisted tomography was performed to confirm the diagnosis if intracranial bleeding was suspected. All cardiac-related adverse events were to be reported. Specific questions were asked about the occurrence of "angina without reinfarction," "angina, presumably reinfarction" and "confirmed reinfarction." Standard 12-lead electrocardiograms (ECGs) were recorded on admission and immediately before coronary angiography. A central analysis of inclusion ECGs was performed to confirm adherence to ECG inclusion criteria. Follow-up examinations took place at 3, 6 and 12 months. At all three follow-up visits the patients' functional state was classified according to the New York Heart Association functional score for heart failure and Canadian Cardiovascular Society score for angina pectoris, both ranging from I to IV. The ECGs were recorded at each visit. Reinfarctions and deaths were documented.

Statistical analysis. Because the study was designed as a pilot trial, no testable hypothesis was formulated. The patency of the infarct-related coronary artery, frequency of bleeding events and effects on hemostatic variables were analyzed as equivalent end points. Two hundred patients were considered sufficient to detect a difference in patency rates of 65% versus 82% (alpha 0.05, beta 0.2). The two groups of patients studied differed only in the prelysis administration of a heparin, or placebo to heparin, bolus. To detect poor efficacy (low patency rate) in the PSH group as early as possible, a sequential analysis of the patency rates, using discordant pairs, formed within each center, of patients from the two treatment groups was employed. This sequential analysis was based on the study of subsequent pairs of patients on different treatments within each center with only pairs discordant concerning patency being used. An interim analysis was scheduled midway through the study. For this analysis, patency rates were compared by

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Table 1. Baseline Characteristics

	HSH Group (n = 56)	PSH Group (n = 62)	95% Confidence Limits of Difference (normal approximation)
Age (yr)	58.6 (9.0)	59.5 (9.2)	(−4.2; 2.4)
Male (%)	83.9	83.9	(−13.6; 13.6)
Weight (kg)	77.7 (11.6)	77.6 (11.1)	(−0.9; 4.5)
Height (cm)	172.4 (7.3)	170.6 (7.7)	(−4.0; 4.2)
Infarct location (%)			
Anterior	46.4	41.9	(−13.8; 22.9)
Inferior	51.8	56.5	(−18.2; 18.8)
Unknown	1.8	1.6	(−4.5; 5.9)
Systolic blood pressure (mm Hg)	130 (21)	136 (21)	(−13.6; 1.6)
Heart rate (beats/min)	74 (17)	77 (19)	(−9.5; 3.5)
Onset of symptoms to start of saruplase infusion (h:min)	2:37 (1:16)	3:08 (1:12)	(−0:57; −0:04)
Time from heparin/placebo bolus to coronary angiography (h:min)	8:22 (2:27)	8:01 (2:27)	(−0:32; 1:14)

Where applicable, data are expressed as mean value (SD). HSH = heparin-saruplase-heparin; PSH = placebo-saruplase-heparin.

the Fisher exact test (alpha 0.05, two-sided). All data were collected and analyzed centrally. An intensive query system was used for missing or inconsistent data. Documentation and study monitoring were performed according to good clinical practice standards. The categoric variables are reported as percentage rates; mean values and standard deviations are used to describe continuous variables. Differences between treatment groups are reported by 95% confidence intervals (CIs).

Results

Interim analysis. After inclusion of the first 100 patients (March 1990) the results of the interim analysis were presented to the central ethics and advisory board. On the basis of this report the central ethics and advisory board decided on July 25, 1990 to terminate the study. By this time a total of 118 patients had been included in the trial, the results of which are presented in the present report.

Baseline characteristics and protocol compliance. Between March 19, 1989 and June 30, 1990, 118 patients were enrolled in the study (56 in the HSH group, and 62 in the PSH group). The two groups of patients were similar with respect to age, gender, height, weight, site of infarction and heart rate (Table 1). A difference of 31 min was found between the time of onset of symptoms and start of thrombolytic infusion; and minor differences were found for systolic blood pressure, fibrinogen and fibrin degradation products. One patient in the PSH group had a previous infarction at the same location. In one patient in the HSH group, typical acute myocardial infarction symptoms were not confirmed. All other patients satisfied the inclusion criteria, and no patient had criterion for exclusion. In 114 patients (54 in the HSH group, 60 in the PSH group), myocardial infarction was confirmed electrocardiographically or enzymatically. A definite diagnosis was not available, in four patients. One patient in the PSH group died early and therefore did not undergo angiography. Ten patients (four in the HSH group, six in the PSH group) underwent coronary angiography after the 6- to 12-h time window. Therefore, 11 patients were classified as having occluded infarct-related coronary arteries for the intention-to-treat analysis.

Angiographic findings. In 117 patients, coronary angiography was performed a mean of 8 h 22 min (±2 h 27 min) after the initial heparin bolus in the HSH group and 8 h 1 min (±2 h 27 min) after the initial placebo bolus in the PSH group. Perfusion (TIMI flow grade 2 or 3) was present in 83.9% of HSH group patients and 62.9% of PSH group patients. An intention-to-treat analysis resulted in patency rates of 78.6% in the HSH group and 56.5% in the PSH group (p = 0.01) (Table 2). The initial time difference between onset of symptoms and start of therapy did not explain the difference in patency rates.

Clinical course. Between the end of catheterization and the end of hospital surveillance, 38 patients underwent percutaneous transluminal coronary angioplasty and 5 coronary

Table 2. Angiographic Findings

Perfusion Grade	HSH Group (n = 56)	PSH Group (n = 62)	p Value
Per protocol analysis (according to angiography performed)			
TIMI flow grade 0 + 1	16.1%	35.5%	
TIMI flow grade 2 + 3	83.9%	62.9%	0.02
Missing	0%	1.6%	
Intention-to-treat analysis (angiograms outside time window were classified as occluded)			
TIMI flow grade 0 + 1	21.4%	43.5%	
TIMI flow grade 2 + 3	78.6%	56.5%	0.01

Data presented are percent of patients. TIMI = Thrombolysis in Myocardial Infarction; other abbreviations as in Table 1.

Table 3. Intervention After 6- to 12-h Angiography

Intervention	HSH Group (n = 56)	PSH Group (n = 62)
PTCA immediately after angiography	17 (30.4%)	21 (34.4%)
Patency after PTCA		
TIMI flow grade 0 + 1	5.9%	19.1%
TIMI flow grade 2 + 3	94 1%	80.9%
Emergency coronary bypass surgery	3 (5.4%)	2 (3.3%)
Intracoronary thrombolysis with urokinase (500,000 U/30 min)	4 (7.1%)	6 (9.7%)

Data presented are number or percent of patients. PTCA = coronary angioplasty; other abbreviations as in Tables 1 and 2.

artery bypass graft surgery. The patency rates after angioplasty were 94.1% (HSH group) and 80.9% (PSH group). Ten patients received intracoronary thrombolysis with urokinase after angiography (Table 3). Bleeding events were reported for eight patients (14.3%) in the HSH group and five (8.1%) in the PSH group. Only one patient (HSH group) required transfusion because of a puncture site hemorrhage (Table 4). There was no marked difference between the two treatment groups with respect to incidence of recurrent infarction, arrhythmias, heart failure and angina pectoris at discharge. Definitive reinfarction occurred during the hospital phase in three patients (5.4%) in the HSH group and in six (9.7%) in the PSH group. No confirmed cerebrovascular event occurred in any of the 118 patients treated. Cerebral bleeding was suspected to be one of the possible causes of convulsion, circulatory collapse and subsequent death of one patient in the PSH group; however, the more likely cause of death was reinfarction or ventricular free wall rupture. An autopsy could not be performed. During the hospital phase there were three deaths (5.4%) in the HSH group and nine (14.5%) in the PSH group. The cause of death was cardiac related in 11 patients (heart failure) and unknown in 1 (PSH group). There was no suggestion or evidence of any allergic reaction occurring during the hospital phase in any of the patients treated.

Cardiac enzymes. The CK-MB peak concentrations and area under the curve were the same in both groups. However, the time to CK-MB peak concentration was shorter in the HSH group than in the PSH group (9 h 33 min [±5 h 36 min] vs. 10 h 39 min [±5 h 46 min], respectively) (Table 5).

Coagulation and fibrinolytic analysis. There was a decline of fibrinogen concentrations as a result of thrombolytic therapy, which was similar in both treatment groups (median [25%, 75% percentile]): from 3.6 (2.8, 4.5) to 0.6 (0.1, 1.7) g/liter in the HSH group and from 3.1 (2.7, 4.0) to 0.9 (0.1, 1.8) g/liter in the PSH group. As expected, the concentrations of fibrinogen and fibrin degredation products increased similarly in both groups. The median thrombin–antithrombin III complex values in the HSH group after lysis were lower than those in the PSH group (Table 6).

Follow-up. Follow-up data were available for 103 patients at 1 year. Twelve patients died during the hospital phase and three during the 1-year follow-up period (cardiac-related events in two [PSH group], unknown events in one [HSH group]). The total mortality rate after 1 year was 12.7% (15 of 118 patients). In the HSH group, the total mortality rate after 1 year was 7.1% (4 of 56 patients), and that for the PSH group was 17.7% (11 of 62). The 1-year reinfarction rate after discharge was 1.9% for both groups. During the 1-year follow-up, five patients underwent bypass surgery and eight coronary angioplasty (four in each group). Health status according to Canadian Cardiovascular Society and functional classifications was similar in both groups after 1 year.

Discussion

The LIMITS study evaluated the 6- to 12-h patency of the infarct-related coronary artery rather than left ventricular function or survival, which are the ultimate goals of thrombo-

Table 4. Adverse Events During Hospital Stay

Adverse Event	HSH Group (n = 56)	PSH Group (n = 62)	95% Confidence Limits of Difference (normal approximation)
Bleeding complications			
All	8 (14.3%)	5 (8.1%)	(−5.0; 17.8)
Puncture site	5 (8.9%)	4 (6.5%)	
Others	2 (3.6%)	1 (1.6%)	
Requiring transfusion	1 (1.8%)	0	
Cardiac events			
Angina, possible reinfarction	1 (1.8%)	4 (6.5%)	
Definite reinfarction	3 (5.4%)	6 (9.7%)	(−14.0; 5.4)
AV block, 2nd or 3rd degree	8 (14.3%)	5 (14.5%)	
Ventricular tachycardia or fibrillation	17 (30.4%)	11 (17.7%)	
Heart failure at discharge	8 (14.3%)	12 (19.4%)	
Angina pectoris at discharge	10 (17.9%)	12 (19.4%)	
Stroke	0	0	
Death	3 (5.4%)	9 (14.5%)	(−20.0; 1)
Allergic reaction	0	0	

Data presented are number (%) of patients. AV = atrioventricular block; other abbreviations as in Table 1.

Table 5. Infarct-Specific Enzyme Activity

	HSH Group (n = 56)	PSH Group (n = 62)	95% Confidence Limits of Difference (normal approximation)
Pretreatment CK-MB (U/liter)	10.8 (7.3)	11.6 (11.2)	(−4.2; 2.6)
Maximal CK-MB (U/liter)	71.8 (58)	71.1 (47.2)	(−18.4; 19.6)
Time from onset of symptoms to maximal CK-MB (h:min)	9:33 (5:36)	10:39 (5:46)	(−3:09; 0:57)
CK-MB area under the curve (U/liter × h)	1,377 (828)	1,384 (830)	(−305; 293)

Data presented are mean value (SD), unless otherwise indicated. CK-MB = creatine kinase-MB isoenzyme; other abbreviations as in Table 1.

lytic therapy after acute myocardial infarction, although the GUSTO (28) study shows that patency can be regarded as a valid end point and predictor of survival. The major finding of this double-blind trial was that treatment with heparin followed by saruplase led to higher coronary patency (78.6%) after 6 to 12 h, whereas treatment with placebo followed by saruplase resulted in a patency rate of only 56.5% (p = 0.01).

Conjunctive thrombolytic therapy. Current thrombolytic strategies in acute myocardial infarction have many shortcomings, including failure of recanalization within 90 min in 15% to 50% of patients (17); delay in the restoration of coronary flow (18); development of reocclusion in 5% to 15% of patients (7); and bleeding complications, especially intracranial bleeding, in up to 0.5% of patients (19).

Conjunctive heparin in mortality trials. The earliest study to evaluate heparin as an adjunctive treatment to thrombolysis was the SCATI study (20), in which thrombolytic therapy with streptokinase was given within 6 h after onset of acute myocardial infarction. The mortality rate was significantly lower (4.5%) in patients randomly assigned to receive postlysis heparin (2,000 U intravenously, followed by 12,500 U subcutaneously every 12 h) compared with the control group (8.8%) with no heparin treatment after thrombolytic therapy. In the GISSI-2 international trials (21,22), the same trend was seen among patients who received both streptokinase and heparin (mortality rate was 7.9% vs. 9.2% for streptokinase alone). These data indicate that heparin given subcutaneously two times/day beginning 9 to 12 h after streptokinase therapy, with or without an intravenous bolus of heparin, is a useful adjuvant to streptokinase treatment in patients with an acute myocardial infarction (23). In the ISIS-2 trial (24), patients were randomized to receive streptokinase or no thrombolysis. Heparin treatment was not part of the study protocol but was used at the discretion of the physician and recorded. Patients with heparin (either subcutaneously or intravenously) in addition to streptokinase had a lower mortality rate (8.7%) than those without heparin (10.1%), but these results may be biased because there was no randomization. The ISIS-3 trial (25) randomized patients with suspected acute myocardial infarction, using a factorial design, to streptokinase, anistreplase or recombinant tissue-type plasminogen activator (rt-PA [duteplase]) and to receive 12,500 U of heparin two times/day subcutaneously, begun 4 h after lysis, or no heparin. All patients were to receive aspirin. During the heparin treatment period, up to day 7 there were slightly fewer deaths for aspirin plus heparin, but there was no significant difference in the 35-day mortality rate for aspirin plus heparin (10.3%) versus aspirin alone (10.6%) (26). In the GISSI-2 international study group trial (21,22), the mortality rate among patients who received rt-PA and heparin (12,500 U subcutaneously twice daily, starting 12 h after lysis) was 9.2% and 8.7% among those who received rt-PA without heparin (p = 0.39). Finally, the addition of subcutaneous heparin to the thrombolytic regimens with streptokinase or rt-PA did not significantly reduce mortality compared with nonuse of heparin (27). In the GUSTO trial (28), there was no difference in mortality rates after streptokinase treatment with intravenous or subcutaneous heparin, but the accelerated rt-PA regimen combined with intravenous heparin (bolus dose of 5,000 U followed by a continuous infusion of 1,000 U/h) resulted in a 14% reduction in mortality rate to 6.3% compared with that after streptokinase. The accelerated rt-PA regimen with intravenous heparin also resulted in better patency rates (29).

Table 6. Coagulation and Fibrinolytic Variables

	HSH Group (n = 56)	PSH Group (n = 62)
Fibrinogen (g/liter)		
Before lysis	3.6 (2.8; 4.5)	3.1 (2.7; 4.0)
End of saruplase infusion	0.6 (0.1; 1.7)	0.9 (0.1; 1.8)
Fibrinogen degradation products (mg/liter)		
Before lysis	0.5 (0.4; 0.7)	0.4 (0.4; 0.5)
End of saruplase infusion	57.0 (13.0; 174.0)	29.5 (2.5; 230.0)
Fibrin degradation products (mg/ml)		
Before lysis	0.5 (0.4; 0.7)	0.4 (0.3; 0.5)
End of saruplase infusion	13.0 (7.3; 17.0)	10.0 (7.0; 15.0)
Thrombin–antithrombin III complex (μg/liter)		
Before lysis	14.6 (6.2; 43.0)	6.6 (3.1; 21.5)
End of saruplase infusion	22.0 (12.0; 70.0)	66.0 (44.0; 142.0)

Data presented are median (25%; 75% percentile). Abbreviations as in Table 1.

Conjunctive heparin in patency trials (Table 7). The earliest randomized controlled study evaluating the additive effect of heparin on coronary patency is the Third Thrombolysis and

Table 7. Heparin in Conjunction With Thrombolysis in Randomized Patency Trials in Acute Myocardial Infarction

Study (ref. no.)	Thrombolytic Agent	Dosage	Heparin Therapy	Control Therapy	Time of Angiography	Patency Rate (no. of pts) (TIMI flow grade 2 or 3)		p Value
						Heparin Group	Control Group	
Topol et al. (30)	Alteplase	1.5 mg/kg in 4 h	Bolus of 10,000 U before lysis	—	90 min	79% (50/63)	79% (54/68)	NS
Bleich et al. (31)	Alteplase	100 mg in 3 h	Bolus of 5,000 U within 1st hour, then 1,000 U/h for 3 d	—	Day 3	71% (30/42)	43% (18/42)	0.015
Hsia et al. (32)	Alteplase	100 mg in 6 h	With start of lysis bolus of 5,000 U, then 1,000 U/h for 7 d	Aspirin 80 mg/d	7–24 h	82% (82/100)	52% (48/93)	<0.0001
de Bono et al. (34)	Alteplase	100 mg in 3 h	Bolus of 5,000 U before lysis, then 1,000 U/h up to angiography plus 250–300 mg aspirin IV or PO	250–300 mg aspirin IV or PO	48–120 h	83.4% (221/265)	74.7% (189/253)	<0.02
O'Connor et al. (35)	Anistreplase	30 U in 2–5 min	4 h after lysis 15 U/kg per h for 24 h, 325 mg aspirin/d	325 mg aspirin/d	Day 5	80% (94/117)	73% (83/114)	NS
Present study	Saruplase	80 mg in 1 h	Bolus of 5,000 U before lysis, 30 min after lysis, 15 U/kg per h for 5 d	Placebo bolus 30 min after lysis, 15 U/kg per h for 5 d	6–12 h	78.6% (44/56)	56.5% (35/62)	0.01

d = day; IV = intravenously; PO = orally; pts = patients; ref. = reference.

Angioplasty in Myocardial Infarction (TAMI-3) trial (30), in which 134 patients with acute myocardial infarction of recent onset (<6 h) received rt-PA (1.5 mg/kg body weight over 4 h) with or without an intravenous bolus of 10,000 U heparin. Coronary angiography was performed 90 min after start of therapy, a when the infusion of rt-PA is still in progress, and showed a patency rate of 79% in both groups. It was concluded that early intravenous heparin does not facilitate the fibrinolytic effect of rt-PA, and heparin therapy can be delayed for at least 90 min after the beginning of thrombolysis with rt-PA. In contrast, in a trial by Bleich et al. (31), 84 patients were treated with 100 mg rt-PA for 3 h and were simultaneously randomized to receive intravenous heparin (bolus of 5,000 U, followed by 1,000 U/h for 3 days) or no heparin. Although coronary angiography was performed on day 3, the patency rate was significantly higher in the heparin (71%) than in the nonheparin group (43%). The Heparin Aspirin Reperfusion Trial (HART) (32,33) compared rt-PA plus heparin with rt-PA plus aspirin in ~200 patients. Patency measured by coronary angiography at a mean of 18 h after start of treatment was 82% in the heparin group and 52% in the aspirin group ($p < 0.0001$). In the Sixth European Cooperative Study Group (ECSG-6) trial (34), all patients received 100 mg of rt-PA and aspirin and were randomized to receive heparin or no heparin up to coronary angiography, which was performed between days 2 and 5. Patency (TIMI flow grade 2 and 3) was present in 83% of patients who had heparin therapy and in 75% of patients who did not ($p < 0.02$). The Duke University Clinical Cardiology Study (DUCCS-1) (35) was a randomized trial to evaluate the effects of heparin (15 U/kg per h, starting 4 h after lysis) in conjunction with anistreplase on coronary patency. A patent infarct-related artery (TIMI flow grade 2 or 3) was present in 80% of patients with and in 73% of those without heparin. At late, predischarge angiography on hospital day 5 the difference was not significant. In general, these studies show that early heparin therapy during the first hours after the start of thrombolytic therapy results in higher patency rates. It is possible that the effect of heparin is different for the various thrombolytic agents.

In the present study, the only difference in postinfarction treatment was the prethrombolytic heparin bolus before saruplase. Whether the difference observed was due to the potentiation of action by saruplase or prevention of rethrombosis remains to be established. However, for alteplase the effect of heparin is more likely to be a reduction in the reocclusion rate (36), and heparin may be replaced with an oral antiplatelet regimen 24 h after thrombolysis (37).

Coagulation and fibrinolytic variables. The changes seen in fibrinogen and in fibrin and fibrinogen degradation products were as expected, and the initial bolus of heparin did not appear to exert an influence on their concentrations. However, pretreatment with heparin may have prevented the increase in thrombin–antithrombin III complex levels seen after saruplase therapy, possibly by preventing the positive feedback production by platelets stimulated during thrombolytic therapy or by inhibiting clot-localized thrombin activity reexposed by thrombolysis.

Bleeding complications. A moderate excess of bleeding was reported in the heparin group (14.3%) compared with that in the placebo group (8.1%), but only one severe bleeding event occurred in each group. Because there was no marked difference in the fibrinogen levels between the groups, this cannot be attributed to a possible increase in nonspecific lytic action by saruplase. There may have been an increase in the overall lytic efficacy caused by heparin cotreatment due to the attenuation of the opposing thrombotic activity of thrombin, as

demonstrated by the reduced TAT III levels in the heparin group. This reasoning is not invalidated by the excess of mild bleeding events in the heparin group because these predominantly took place at puncture sites.

Limitations of the study. Antiplatelet drugs were not allowed during the first 5 days of the study. Higher patency rates, especially in the PSH group, might have been seen had these drugs been given. However, although mortality rate is lowered with aspirin (24), to our knowledge there has been no study to test the effect on patency rate. At the time of the design of the present study, the accepted definition of an open vessel was TIMI flow grade 2 or 3. A retrospective analysis of our data has shown that TIMI flow grade 3 was 75.0% (HSH group) and 59.6% (PSH group). Although the magnitude of the patency rate is diminished, the clinical impression remains the same. Patients in the HSH group were treated on average 31 min earlier, and it may be assumed that this contributed to the difference in patency rates. The patency rate for those patients treated within 2 h in the HSH group was 86.4% versus 84.2% for those treated between 2 and 3 h. For the PSH group these were 50.0% and 66.7%, respectively. Therefore, although there is a difference with time, this does not appear to have played a major role in the result.

Clinical implications. The present study has important implications for the clinical use of saruplase because it shows that heparin given before the start of saruplase is necessary for improved efficacy (although there is a slight tendency toward more bleeding). Although the purpose of this pilot trial was not to recruit sufficient numbers of patients to demonstrate intergroup differences in mortality rate, the marked increase in the patency rate in the heparin group may have translated into the observed trend to a reduced in-hospital mortality rate (5.4% vs. 14.5% in the placebo group) (38). On the basis of our findings that heparin pretreatment further improved the thrombolytic efficacy of saruplase without markedly increasing the incidence of severe adverse events, it must now be strongly urged that saruplase treatment always be preceded by a heparin bolus (5,000 IU intravenously).

Appendix

LIMITS Study Group

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